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**Prostate Cancer Evaluation: Design, Synthesis and Evaluation of
Novel Enzyme-Activated Proton MRI Contrast Agents**

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14. ABSTRACT The lacZ gene encoding E. coli beta-gal has already been recognized as the most commonly used reporter system in cancer gene therapy. Moreover, prostate-specific membrane antigen (PSMA) has been identified as an ideal antigenic target in prostate cancer. We propose to develop a novel class of Gd(III)-based MRI contrast agents for in vivo detection of beta-gal or PSMA activity. This new concept of the Gd(III)-based MRI contrast agents is composed of three moieties: (A) a signal enhancement group, such as Gd-DOTA or Gd-PCTA; (B) an Fe(III) chelating group; (C) beta-D-galactose or glutamate. Following cleavage by lacZ transgene or PSMA in prostate cancer cells, the released, activated aglycone Fe(III)-ligand will spontaneously trap endogenous Fe(III) at the site of enzyme activity forming a highly stable complex, to restrict motion of the Gd(III) chelates enhancing relaxivity and providing local contrast accumulation. We plan to synthesize 8 novel MRI contrast agents for imaging beta-gal or PSMA activity in prostate cancer cell culture, explore the feasibility of applying the most promising analogies to cells grown in vivo in mice and rats.												
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INTRODUCTION

Prostate cancer is the most frequently diagnosed cancer and the leading cause of cancer death in men in the United States, an estimated 218,890 new cases with 27,050 deaths in 2007.[1,2] Gene therapy has emerged as a potentially promising strategy for treatment of prostate cancer.[3-15] The prostate is particularly amenable to gene therapy.[11-16] However, there are major issues in terms of assessing the delivery to target tissue, assessing the uniformity (versus heterogeneity) of biodistribution and determining whether the genes are expressed.[15-33] A viral construct is often readministered on successive occasions, but this should optimally be timed to coincide with loss of expression. Inevitably gene therapy has associated risks, and thus non-invasive *in vivo* determining the duration of gene expression in an individual tumor could greatly enhance the viability of the approach.

Gene expression now is commonly monitored by *in situ* hybridization techniques or by introducing a marker gene to follow the regulation of a gene of interest. Since β -galactosidase (β -gal) activity is readily assessed by histology or in culture, in hosts as evolutionarily diverse as bacteria, yeast, and mammals, its introduction has become a standard means of assaying clonal insertion, transcriptional activation, protein expression, and protein interaction, *lacZ* gene encoding *E. coli* β -gal has already been recognized as the most commonly used reporter system.[34] However, the well-established chromogenic or fluorogenic substrates, relying on the hydrolysis by β -gal to release colorful compounds are limited to histology or *in vitro* assays.[35-39] Non-invasive *in vivo* detecting of transgene expression would be of considerable value in many ongoing and future clinical gene therapy trials.

The superb spatial resolution and the outstanding capacity of differentiating soft tissues have determined the widespread success of magnetic resonance imaging (MRI) in clinical diagnosis.[40] The contrast in an MR image is the result of a complex interplay of numerous factors, including the relative T_1 and T_2 relaxation times, proton density of the imaged tissues and instrumental parameters. It was shown that contrast agent causes a dramatic variation of the water proton relaxation rates, thus providing physiological information beyond the impressive anatomical resolution commonly obtained in the uncontrasted images. Contrast agents are widely used clinically to assess organ perfusion, disruption of the blood-brain barrier, occurrence of abnormalities in kidney clearance, and circulation issues.[40-44] The responsive MRI contrast agents holds great promise in the gene therapy arena.[45,46] The abilities of these contrast agents to relax water protons is triggered or enhanced greatly by recognition of a particular biomolecule opening up the possibility of developing MRI tests specific for biomarkers

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indicative of particular disease states and aiding in the early detection and diagnosis of tumors. Desreux *et al* [42,47] demonstrated that, by chelating $\text{Gd}(\text{phen})\text{HDO3A}$ with $\text{Fe}(\text{II})$ to form a highly stable tris-complex, as shown in **Figure 1**, the relaxivity increased 145% at 20MHz and 37°C from $5.1\text{mM}^{-1}\text{s}^{-1}$ per $\text{Gd}(\text{III})$ in $\text{Gd}(\text{phen})\text{HDO3A}$ form to $12.2\text{mM}^{-1}\text{s}^{-1}$ in the tris-complex. Desreux *et al* [42,47] also synthesized another iron-sensitive MRI contrast agent with a tris-hydroxamate (**Figure 2**). After the tris-hydroxamate groups formed a chelate with $\text{Fe}(\text{III})$, free rotation at the $\text{Gd}(\text{III})$ centers was restricted, thereby increasing relaxivity by 57% from 5.4 to $8.5\text{mM}^{-1}\text{s}^{-1}$ at 20 MHz.

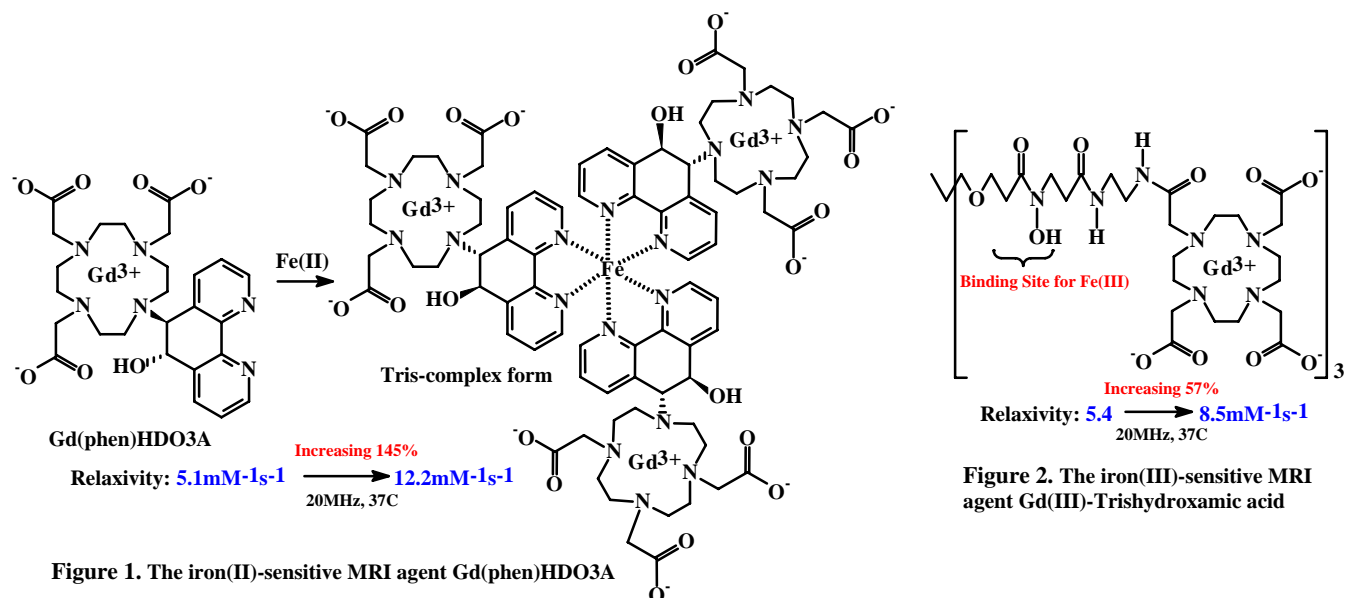


Figure 1. The iron(II)-sensitive MRI agent $\text{Gd}(\text{phen})\text{HDO3A}$

Figure 2. The iron(III)-sensitive MRI agent $\text{Gd}(\text{III})$ -Trishydroxamic acid

Iron is a critically important metal ion for a wide variety of cellular events.[48] Tumor cells, as compared with their normal counterparts, frequently exhibit increased uptake and utilization of iron, as evidenced by an increase in transferrin receptors at the cell surface.[49-51] Additionally, cancer cells are sensitive to the effects of iron chelators because of the critical requirement for iron in proteins that play essential roles in DNA synthesis and energy production.[52,53] Such studies have led to iron chelation therapy to clinically treat some tumors.[54-58]

Based on the MRI contrast agents findings and the biologic features of tumor, we have proposed in this project a novel class of enzyme activated Gd^{3+} -based MRI contrast agent for *in vivo* detection of β -gal activity, in which we try to combine all means of reaching the highest possible relaxivities (**Figure 3**).[42,47]

Additionally, prostate-specific membrane antigen (PSMA) is a type II transmembrane glycoprotein with enzymatic activities: N-acetylated α -linked L-amino dipeptidase (NAALADase) and γ -glutamyl

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carboxypeptidase (folate hydrolase).[59-61] Studies with the monoclonal antibodies have demonstrated that PSMA is the most well-established, highly restricted prostate cancer cell surface antigen, it is expressed at high density on the cell membrane of all prostate cancers.[62-64] The high prostate tissue specificity of PSMA has been identified as an ideal therapeutic and diagnostic target for prostate cancer, this potential was exemplified by the recent FDA approval of an ^{111}In -labeled PSMA monoclonal antibody (Prostascint[®]) for diagnostic imaging of prostate cancer.[65-67] Furthermore, phase I and II trials have begun using immunotherapy directed against PSMA.[68-70] By introducing γ -glutamate residue instead of D-galactose in our proposed above new mechanism diagram, we intend to develop a novel class of Gd(III)-based MRI contrast agents for *in vivo* imaging prostate tumor through PSMA activated *in situ* Fe^{3+} -trapped MRI contrast agent formation (**Figure 4**).

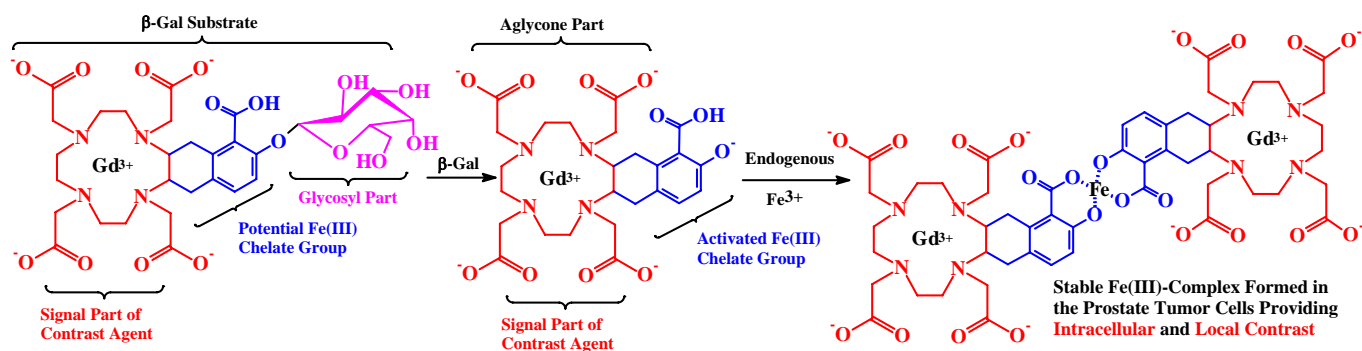


Figure 3. Mechanism of proposed new platform for *in vivo* detection of *lacZ* gene expression through β -gal activated *in situ* Fe^{3+} -trapped MRI contrast agent formation.

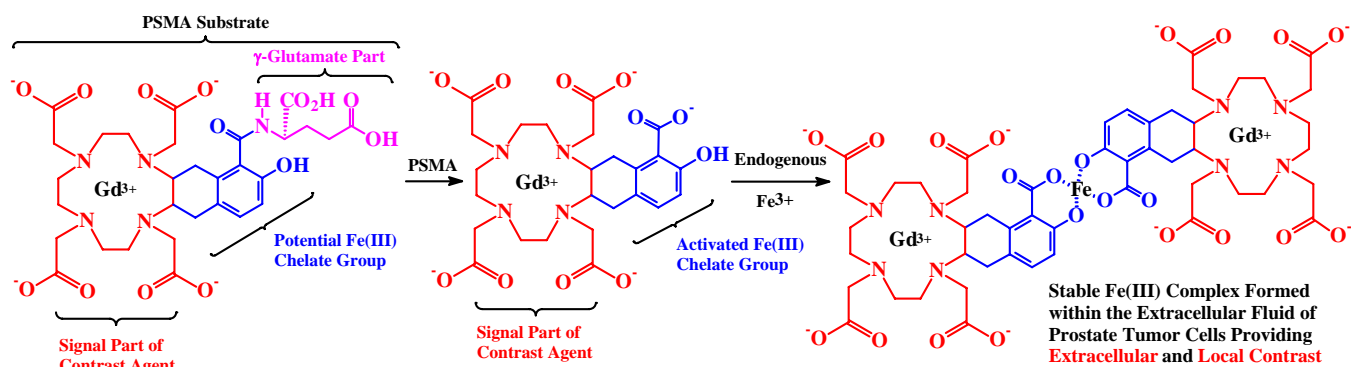


Figure 4. Proposed new mechanism for *in vivo* imaging prostate tumor through PSMA activated *in situ* Fe^{3+} -trapped MRI contrast agent formation.

Especially, PSMA has a large extracellular domain,[70] so the expression of PSMA tethered to the surface of the prostate cancer cells makes that the novel peptide-based MRI contrast agents can be targeted for activation within the extracellular fluid of prostate cancers [71] and overcomes the need for a peptide-based MRI contrast agent to penetrate the tumor cell membrane, thus, providing *in vivo* prostate

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cancer imaging through an **extracellular** MRI approach. The concern of permeability is one of the greatest challenges in the development of *in vivo* MRI contrast agents.[72]

Accordingly, depending upon the enzyme sources either being the *lacZ* transgene or the PSMA from prostate tumors, this new platform could provide *in vivo lacZ* gene expression assay or *in vivo* prostate cancer imaging (in particular, through **extracellular** contrast agents), with combining all the approaches of reaching the highest possible relaxivities.[42,47,72] Furthermore, this new class of responsive MRI contrast agent is composed of three functional moieties, in which the signal enhancing and Fe^{3+} chelating parts are flexible allowing modification in a search for ideal Fe^{3+} -trapped MRI contrast agents. Importantly, the combination of three functional moieties is based on the clinically applied strategies on cancer therapy. These facts strongly suggest the potential of the proposal to future clinical application.

Most recently, Merbach *et al* [73-76] observed the remarkably high T_1 relaxivity gain by the heterometallic, self-assembled metallostear formation with six efficiently relaxing Gd^{III} centers from $(\text{tpy-DTTA})\text{Gd}(\text{H}_2\text{O})$ with $7.3\text{mM}^{-1}\text{s}^{-1}$ to $\{\text{Fe}^{\text{II}}[\text{Gd}^{\text{III}}_2(\text{tpy-DTTA})_2(\text{H}_2\text{O})_4]_3\}^{4-}$ with $15.7\text{mM}^{-1}\text{s}^{-1}$ at 20MHz and 37°C (**Figures 5**), significantly, their detailed studies on structure and dynamics of the trinuclear complex $\{\text{Fe}^{\text{II}}[\text{Gd}^{\text{III}}_2(\text{tpy-DTTA})_2(\text{H}_2\text{O})_4]_3\}^{4-}$ indicate that the heterometallic self-assemblies attain high T_1 relaxivities by influencing three factors: water exchange, rotation, and electron relaxation, which are fully consistent with the expecting results shown as above in **Figures 3** and **4**, the effectiveness of contrast agents can be increased by restricting the motion of $\text{Gd}(\text{III})$ chelates by linking them rigidly to macromolecules through covalent or non-covalent bonds, by an improvement of their intrinsic relaxivity or by attaching several paramagnetic entities to biological or synthetic oligomers. Obviously, these most recently comprehensive investigations as relevant evidences strongly support for our current proposal.

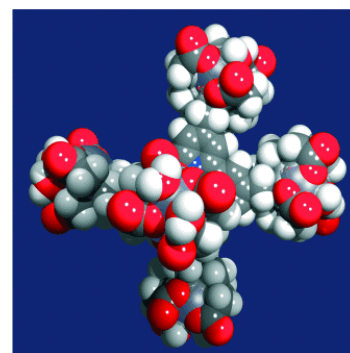


Figure 5. $[\text{Fe}\{\text{Gd}_2\text{L}(\text{H}_2\text{O})_4\}_3]^{4-}$

STATEMENT OF WORK

Specific Aim 1 Design and synthesize model “smart” MRI contrast agents to report β -gal or PSMA activities with the ability of trapping Fe^{3+} ion.

Task 1 Design and optimization of synthetic strategies for reporter molecules. (**Months 1-18**)

Task 2 Structural characterizations of the synthesized molecules. (**Months 4-20**)

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Specific Aim 2 Test the properties of molecules in solution and *in vitro* with cultured prostate cancer cells.

Task 3 Evaluation the basic properties of the agents **in solution**. (Months 20-22)

Task 4 Evaluation of the properties of the optimal molecules *in vitro* **with cultured prostate cancer cells**. (Months 23-25)

Specific Aim 3 Scale up synthesis of the most promising MRI contrast agent(s) and apply to animal investigations.

Task 5 Scale up synthesis of the most promising ^1H MRI contrast agent(s). (Months 26-28)

Task 6 Apply the most promising ^1H MRI contrast agent(s) to assess β -gal transfection efficiency, *lacZ* gene expression (spatial and temporal) in prostate tumors *in vivo* (48 mice + 48 rats). (Months 29-35)

Task 7 Test dosing protocols, timing, MR detection protocols (48 mice) (Months 29-35)

Task 8 Prepare manuscripts and final report (Month 36)

BODY

In this third supported year, our work continued followed the research plan of the approved proposal W81XWH-05-1-0593 on: **Task 1** Design and optimization of synthetic strategies for reporter molecules; **Task 2** Structural characterizations of the synthesized molecules; **Task 3** Evaluation the basic properties of the agents in solution; and **Task 4** Evaluation of the properties of the optimal molecules *in vitro* with cultured prostate cancer cells; **Task 5** Scale up synthesis of the most promising ^1H MRI contrast agent(s); **Task 6** Apply the most promising ^1H MRI contrast agent(s) to assess β -gal transfection efficiency, *lacZ* gene expression (spatial and temporal) in prostate tumors *in vivo*; **Task 7** Test dosing protocols, timing, MR detection protocols (48 mice).

In the second supported year, we have successfully obtained the molecules **A**, **B** and **C**, which are very close analogues to the designed target reporters **M₁**, **M₃** and **M₅**, respectively (see **Figure 1**). In this third year supported year, the various methods trying to simultaneously remove the ethyl and galactosyl in ester moieties of **A**, **B** and **C**, while preserving the galactosyl ether part stable, have been explored, however, the attempts were not fruitful to accomplish the desired reporter molecules **M₁**, **M₃** and **M₅**.

Based on these situations, we modified the synthetic strategies and redesigned the synthesis routes: tert-Butyl (instead of Ethyl) and benzyl groups were chosen, since they can be readily and selectively removed. As I mentioned in the second year's progress report, the modified synthetic strategies had been

Reaction scheme for the synthesis of compound 1:

Starting material: 2-naphthol-1-carboxylic acid.

Step 1: 2-naphthol-1-carboxylic acid $\xrightarrow{\text{BnBr, KOH}}$ 2-naphthol-1-carboxylate benzyl ester.

Step 2: 2-naphthol-1-carboxylate benzyl ester $\xrightarrow{\text{Li/NH}_3}$ 1,4-dihydro-2-naphthol-1-carboxylate benzyl ether.

Step 3: 1,4-dihydro-2-naphthol-1-carboxylate benzyl ether $\xrightarrow{1. \text{MMPP}, 2. \text{HAc}}$ 1,4-dihydro-2-naphthol-1-carboxylate benzyl ether with two hydroxyl groups.

Step 4: 1,4-dihydro-2-naphthol-1-carboxylate benzyl ether with two hydroxyl groups $\xrightarrow{\text{MsCl, Py}}$ 1,4-dihydro-2-naphthol-1-carboxylate benzyl ether with two methanesulfonyl groups.

Step 5: 1,4-dihydro-2-naphthol-1-carboxylate benzyl ether with two methanesulfonyl groups $\xrightarrow{\text{NaN}_3, \text{DMF}}$ 1,4-dihydro-2-naphthol-1-carboxylate benzyl ether with two diazo groups.

Step 6: 1,4-dihydro-2-naphthol-1-carboxylate benzyl ether with two diazo groups $\xrightarrow{\text{PPh}_3}$ 1,4-dihydro-2-naphthol-1-carboxylate benzyl ether with two amino groups.

Step 7: 1,4-dihydro-2-naphthol-1-carboxylate benzyl ether with two amino groups $\xrightarrow{\text{bis-chloroacetamide derivative}}$ 1,4-dihydro-2-naphthol-1-carboxylate benzyl ether with two amino groups and a macrocyclic structure.

Step 8: 1,4-dihydro-2-naphthol-1-carboxylate benzyl ether with two amino groups and a macrocyclic structure $\xrightarrow{\text{B}_2\text{H}_6}$ 1,4-dihydro-2-naphthol-1-carboxylate benzyl ether with two hydroxyl groups and a macrocyclic structure.

Step 9: 1,4-dihydro-2-naphthol-1-carboxylate benzyl ether with two hydroxyl groups and a macrocyclic structure $\xrightarrow{\text{complex ester derivative}}$ 1,4-dihydro-2-naphthol-1-carboxylate benzyl ether with two hydroxyl groups and a macrocyclic structure with a benzyl ester and a benzyl ether.

Step 10: 1,4-dihydro-2-naphthol-1-carboxylate benzyl ether with two hydroxyl groups and a macrocyclic structure with a benzyl ester and a benzyl ether $\xrightarrow{\text{Pd/C, H}_2}$ 1,4-dihydro-2-naphthol-1-carboxylate benzyl ether with two hydroxyl groups and a macrocyclic structure with a benzyl ester and a benzyl ether.

Step 11: 1,4-dihydro-2-naphthol-1-carboxylate benzyl ether with two hydroxyl groups and a macrocyclic structure with a benzyl ester and a benzyl ether $\xrightarrow{\text{complex ester derivative}}$ 1,4-dihydro-2-naphthol-1-carboxylate benzyl ether with two hydroxyl groups and a macrocyclic structure with a benzyl ester and a benzyl ether (Compound 1).

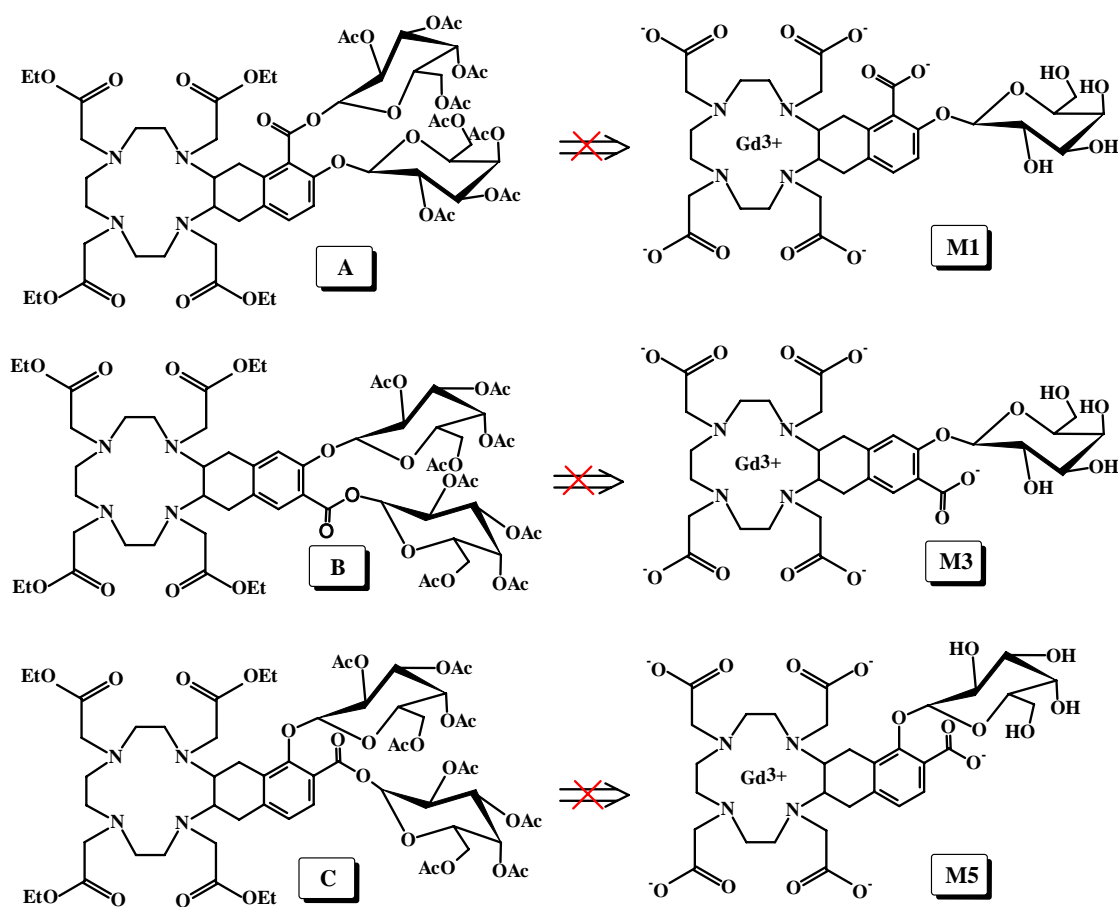


Figure 1. Synthesis of A, B, C and the Structure of M₁, M₃ and M₅

According the following multiple reactions (see **Figure 2**), I eventually achieved the designed target molecules **M**₁, **M**₃ and **M**₅.

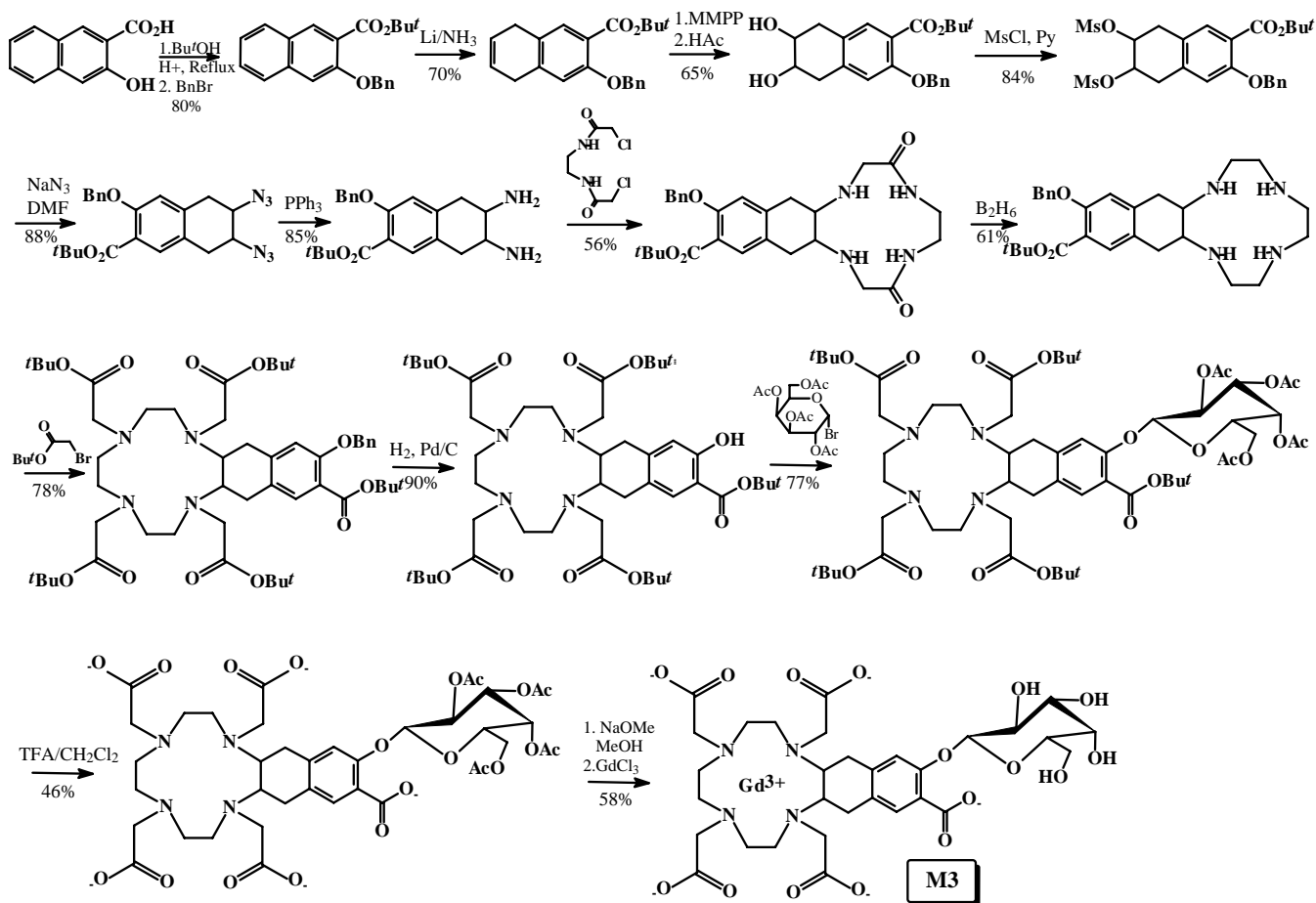
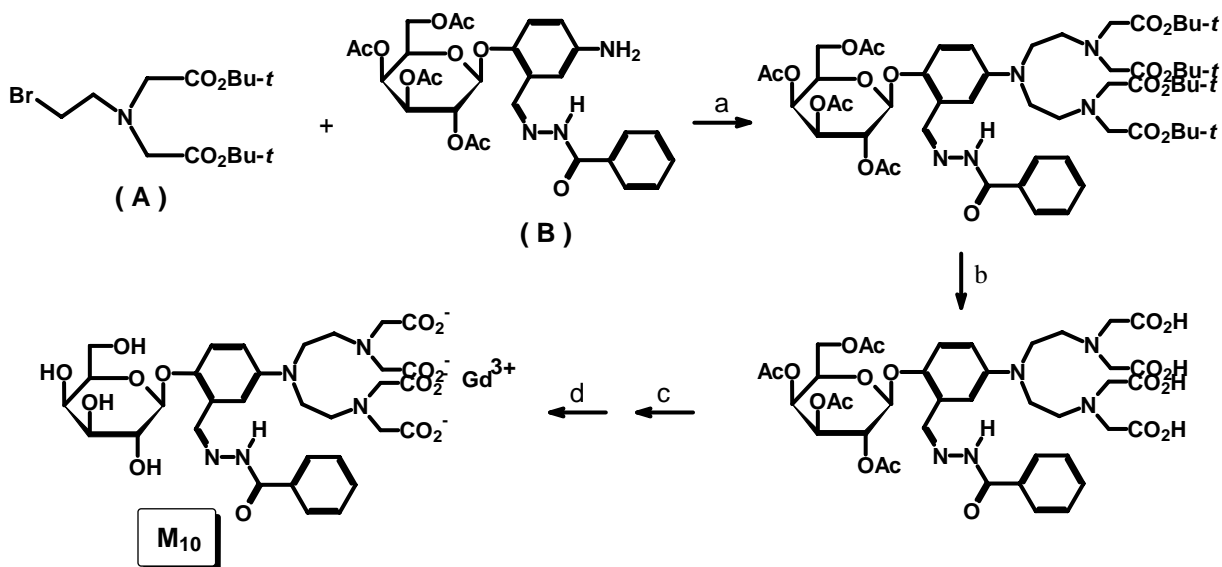
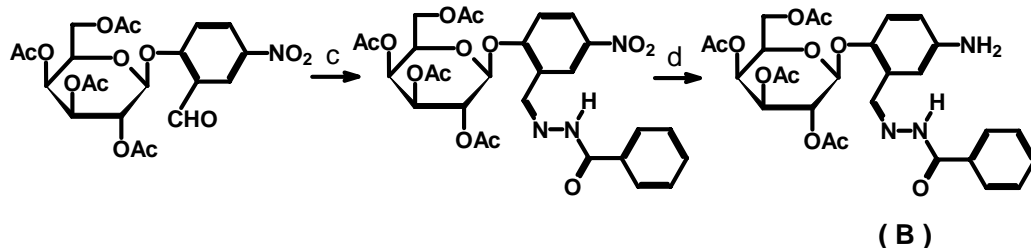


Figure 2.

Meanwhile on struggling for accomplishing the representative target molecules designed in the approved proposal W81XWH-05-1-0593, we also attempted to develop the alternate candidates based on the novel mechanism proposed in W81XWH-05-1-0593, and combining with the most recent findings about MRI contrast agents. Because diethylenetriamine-*N, N, N'*, *N''*-tetraacetate (DTTA) as Gd(III) chelator has been proven to ensure the complex to sufficient thermodynamic stability, a water exchange faster (closer to optimal) than that of commercial agents and two inner sphere water molecules to double the inner sphere contribution to the relaxivity.[40,73] So merging DTTA into our responsive MRI contrast agents instead of DOTA or PCTA as Gd(III) chelating moiety should produce particularly high relaxivity and sensitivity. In the second supported year, we have successfully obtained the molecule **M**₉ with the following merits: (1) DTTA has been proven to ensure the complex to sufficient thermodynamic

Based on this success, I extended this strategy further for the synthesis and achieved another new molecule **M**₁₀, an analogue to the molecule **M**₉, which merges the Fe-chelate antitumor drug structure as the Fe-chelator into the responsive MRI contrast agent (see **Figure 3**).



This part work has been reported on the World Molecular Imaging Congress in Nice, France, Sept. 10-13, 2008.

The MRI evaluation of the reporter molecules **M**₁, **M**₃, **M**₅, **M**₇, **M**₉ and **M**₁₀, respectively, in sodium phosphate buffer solution (PBS) (0.1 M, pH=7.4) in the presence of ferric ammonia citrate (FAC) with β -galactosidase E801A indicated that:

(1) the reporter molecules **M**₁, **M**₃, **M**₅, **M**₇ can not be hydrolyzed by β -galactosidase E801A, so no MRI contrast changes before and after addition of β -galactosidase E801A can be seen.

(2) the reporter molecules **M**₉ and **M**₁₀ can be hydrolyzed by β -galactosidase E801A in the presence of FAC in PBS (0.1 M, pH=7.4), and much big MRI contrast changes before and after reacting with β -galactosidase E801A can be observed (see **Figure 4**), it implies that the released aglycone including the activated Fe^{3+} -ligand and MRI signal enhancement group spontaneously traps Fe^{3+} in the solution forming a highly stable complex, then restricting the motion of the Gd^{3+} chelates enhancing relaxivity.

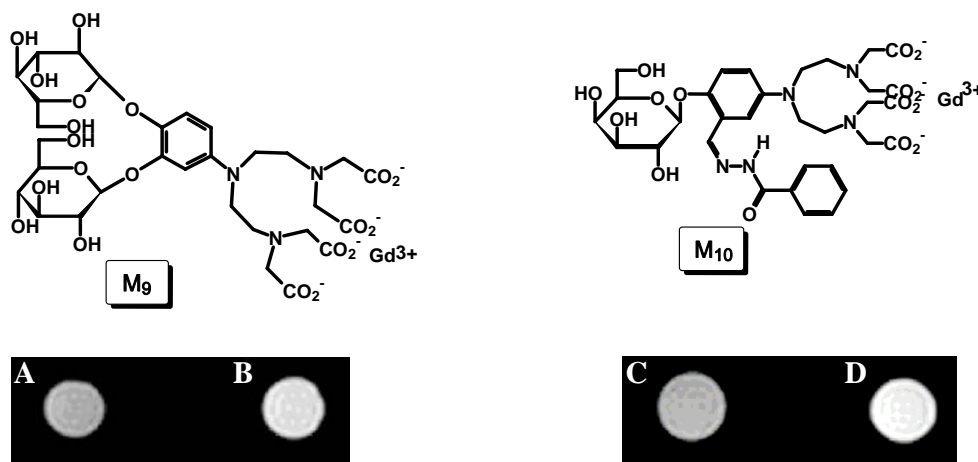


Figure 4. T_1 -weighted (TR/TE 150/12 ms) MR images of solutions and the signal intensity in test tubes at 4.7 T MR scanner: (A) PBS with **M**₉ and FAC; (B) PBS with **M**₉, FAC and β -galactosidase E801A; (C) PBS with **M**₁₀ and FAC; (D) PBS with **M**₁₀, FAC and β -galactosidase E801A.

The further evaluation of **M**₉ and **M**₁₀ with *lacZ* transfected prostate tumor cells is ongoing. Since the additional time and efforts on exploring the syntheses arising from our years 2 and 3 results and failure in obtaining the designed molecules by the initial synthetic strategies and routes have prevented us from finishing our studies in 3 years as proposed. We requested a one-year extension for the project with the remaining funds carried over (no additional cost extension). Considering our progress (years 1-3) in fine defining the reporter molecule structures and the synthetic methodological exploration as well

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as already demonstrated this novel mechanism for *in vivo* prostate cancer imaging and evaluation of prostate cancer gene therapy as described in W81XWH-05-1-0593, we are confident that we will be able to carry out the proposed studies in the remaining time.

RESEARCH ACCOMPLISHMENTS

(1) Modified the synthetic strategies and redesigned the synthesis routes;

(2) Achieved a series of the target molecules **M₁**, **M₃**, **M₅** and **M₁₀**, and verified the structures by NMR data.

(3) Evaluated the reporter molecules **M₁**, **M₃**, **M₅**, **M₇**, **M₉** and **M₁₀**, respectively, in sodium phosphate buffer solution (PBS) (0.1 M, pH=7.4) in the presence of ferric ammonia citrate (FAC) with β -galactosidase E801A, and found that: (a) the molecules **M₁**, **M₃**, **M₅**, **M₇** can not be hydrolyzed by β -galactosidase E801A, so no MRI contrast changes before and after addition of β -galactosidase E801A can be seen; (b) the reporter molecules **M₉** and **M₁₀** can be hydrolyzed by β -galactosidase E801A in the presence of FAC in PBS (0.1 M, pH=7.4), and much big MRI contrast changes before and after reacting with β -galactosidase E801A can be observed, it demonstrated this novel mechanism for *in vivo* prostate cancer imaging and evaluation of prostate cancer gene therapy as described in W81XWH-05-1-0593.

REPORTABLE OUTCOMES

The design and synthesis of target molecule **M₁₀** has been reported on the World Molecular Imaging Congress in Nice, France, Sept. 10-13, 2008.

CONCLUSIONS

Prostate cancer is the most commonly diagnosed cancer and the second most common cause of cancer death in men in the United States. The advent of effective screening measures can sharply decrease the mortality of prostate cancer through detecting this disease at an earlier stage. However, the evidence for mortality benefit from prostate cancer screening has been disappointing to date. Expanding knowledge of prostate cancer biology with combination of imaging technologies would be of considerable value in many ongoing and future clinical prostate cancer diagnosis and gene therapy trials.

Based on the biologic features of prostate cancer, we proposed in this project a new approach for *in vivo lacZ* gene expression assay or *in vivo* prostate cancer imaging. The ultimate objective is to demonstrate the utility and reliability of this new approach to measure β -gal or PSMA activities *in vivo*. We have accomplished a series of target molecules **M₁**, **M₃**, **M₅**, **M₇**, **M₉** and **M₁₀**, and verified by NMR data. Much big MRI contrast changes of target molecules **M₉** and **M₁₀** before and after exposing to β -

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galactosidase E801A demonstrated the novel mechanism for *in vivo* prostate cancer imaging and evaluation of prostate cancer gene therapy as described in W81XWH-05-1-0593. We are now focusing on evaluating the characterization of these two target molecules with *lacZ* transfected prostate tumor cells, and scale up the syntheses based on the modified synthetic strategies, anticipating to apply the most promising MRI contrast agents for the sequence of tests with prostate cancer *in vivo*.

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APPENDICES

- (1) Poster #692 on the World Molecular Imaging Congress in Nice, France, Sept. 10-13, 2008.

DESIGN AND SYNTHESIS OF NOVEL *lacZ* RESPONSIVE ENHANCED MRI AGENT

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UT SOUTHWESTERN MEDICAL CENTER

PRESENTATION # 0653

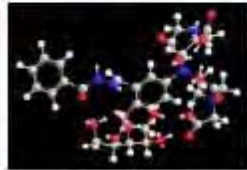
INTRODUCTION

Gene therapy holds great promise for treating cancer and has been successfully exploited in several clinical trials. A major current obstacle to implementation is to establish a method of assessment of therapeutic gene expression in terms of heterogeneity and longevity in tissues. The *lacZ* gene encoding β -galactosidase (β -gal) has been recognized as the most attractive reporter gene. Therefore, noninvasive detection *in vivo* would be of considerable value in future clinical cancer gene therapy trials.¹ The well-established chromogenic or fluorogenic substrates, relying on the hydrolysis by β -gal to release colored compounds, are limited to histology or *in vitro* assays. Weisleder *et al.* presented a near infrared *in vivo* approach based on DDMAC.² Meade *et al.* reported a ^1H MRI approach using FgaMe.³ Recently we have presented ^1H MRI,⁴ ^{19}F MRS and CSI methods using S-pal,⁵ AZD-6 and fluorinated phenolic β -D-galactosides.⁶ We now explore a new class of *lacZ* gene responsive enhanced MRI contrast agents.

DESIGN

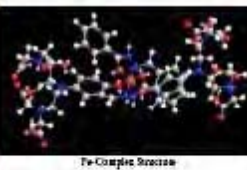
Dumas *et al.* demonstrated that, by chelating Gd(phenyl)HDO₃A with Fe(II), to form a highly stable tri-complex, the relaxivity increased 140% at 20 MHz and 37°C (Figure 1).⁷ More recently, Tish *et al.* showed that the hexamethalate, self-assembled metal ions with six ethylenediamine Gd(III) centers, $[\text{Fe}(\text{Gd}_6\text{L}_6\text{H}_6\text{O}_{18})_4]^{12-}$ (Figure 2), exhibited a particularly high relaxivity for its moderate molecular weight.⁸

STRUCTURAL FEATURES



Ligand Structure

STRUCTURAL FEATURES



Fe-Complex Structure

Figure 1

Design

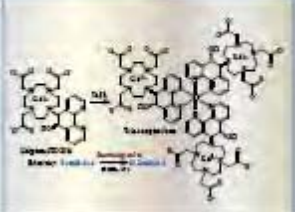


Figure 1: The Iron(II)-sensitive MRI agent Gd(phenyl)HDO₃A

Figure 2




Figure 2: The hexamethalate $[\text{Fe}(\text{Gd}_6\text{L}_6\text{H}_6\text{O}_{18})_4]^{12-}$

DESIGN

Iron is a critically important metal ion for a wide variety of cellular events. Tumor cells frequently exhibit increased uptake and utilization of iron. Additionally, cancer cells are sensitive to the effects of iron chelators because of the critical requirement for iron in proteins that play essential roles in DNA synthesis and energy production. Such studies have led to iron chelation therapy to clinically treat some tumors.

We propose a novel class of enzyme activated Gd³⁺-based MRI contrast agents for *in vivo* detection of β -gal activity combining three features: (A) a signal enhancement group, such as Gd-DOTA; (B) an Fe²⁺ chelating group using the structure of clinical iron chelation therapy drugs; (C) β -galactosidase. Following cleavage by β -gal in cells, the released, activated aglycone Fe²⁺-ligand will spontaneously trap endogenous Fe²⁺ as the site of enzyme activity forming a highly stable complex, exhibiting restricted motion of the Gd³⁺-chelate enhancing relaxivity and providing contrast based on gene (*in vivo* enzyme)-stimulated local accumulation (Figure 3).

Figure 3




Figure 3: The proposed new mechanism for *in vivo* detection of *lacZ* gene expression through Fe-trapped MRI contrast agent formation.

Figure 4

SYNTHESIS




Figure 4: Reaction Conditions: (a) $\text{H}_2\text{NCH}_2\text{CH}_2\text{NH}_2$, H_2O , AcOH , reflux, 62%; (b) $\text{H}_2\text{NCH}_2\text{CH}_2\text{NH}_2$, H_2O , AcOH , reflux, 62%; (c) $\text{H}_2\text{NCH}_2\text{CH}_2\text{NH}_2$, H_2O , AcOH , reflux, 62%.

Figure 5

SYNTHESIS

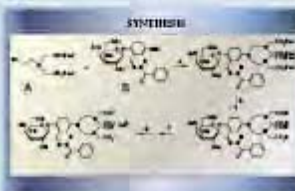
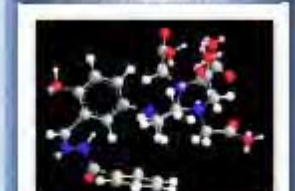


Figure 5: Reaction Conditions: (a) H_2O , MeOH , 42%; (b) $\text{CF}_3\text{CO}_2\text{H}$, CH_2Cl_2 , 44%; (c) GdCl_3 , Pyridine, 79%; (d) MeOH , Me_2SO , 62%.

STRUCTURAL FEATURES



Ligand Structure

CONCLUSIONS

We propose a novel class of enzyme activated Gd³⁺-based MRI contrast agents for detection of β -gal activity. Synthesis of the substrate has been successful, and we are now evaluating this agent with β -gal expressing cells and *in vivo*.

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ADDITIONAL INFORMATION

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